

ON SUCCESSIVE DUPLICATE MUTATIONS.¹

R. RUGGLES GATES,
UNIVERSITY OF LONDON.

Nilsson-Ehle² was the first to formulate the hypothesis of duplicate factors or representatives for the same character. He brought forward evidence from crosses of red and white varieties in certain Swedish strains of wheat, tending to show that in different F_2 families plants with red and white grains occurred in the ratios respectively 3:1, 15:1 and 63:1; from which he concluded that three independent units for red were present, each of which could produce the color alone. Although his conclusions were criticized by Kajanus,³ yet there remains a strong presumption in their favor, and several other cases of supposed duplicate factors have been described, though these have for the most part rested upon more insecure data than the original instances of Nilsson-Ehle.

Certain suggestions have been made concerning the origin of this duplicate or triplicate condition. Emerson and East⁴ pointed out in general terms that if a factor should become located in a different chromosome or should be affected in any way so as not to be always allelomorphic to itself, then a duplication of determiners would result. Shull⁵ has listed the supposed cases of duplicate determiners and remarks that such a condition of duplication might also result from "repeated progressive mutations." In the same paper, Shull endeavors to account for the origin simultaneously of a duplicate "gene"

¹ Presented before the American Genetic Association, San Francisco meeting, August 3, 1915.

² Nilsson-Ehle, H., 1909, "Kreuzungsuntersuchungen an Hafer und Weizen," I., *Lunds Univ. Arsskrift.*, N.F., Afd. 2, Bd. 5, Nr. 2, pp. 122.

³ Kajanus, B., 1914, "Zur Kritik des Mendelismus," *Zeitschr. f. Abst. u. Vererb.*, 12: 206-224.

⁴ Emerson, R. A., and East, E. M., 1913, "The Inheritance of Quantitative Characters in Maize," *Agric. Exp. Sta. Nebraska, Research Bull.* 2, pp. 120.

⁵ Shull, Geo. H., 1914, "Duplicate Genes for Capsule-form in *Bursa bursa-pastoris*," *Zeitschr. f. Abst. u. Vererb.*, 12: 97-149, Figs. 7.

for capsule form in *Bursa bursa-pastoris* and, at the same time, of the mutant *B. Heegeri*. That hypothesis will not, however, apply to the probably more frequent cases in which duplicate factors for a particular character are found without any other mutation having taken place. An explanation will therefore have to be found for the duplicate or triplicate condition in wheat or in any other organisms in which it occurs.

It is the purpose of the present paper to discuss more precisely the manner in which such monohybrid characters originate and particularly the way in which they may afterward become duplicate or triplicate. *Oenothera rubricalyx* affords a typical case of a mutant originating as a monohybrid, probably through a transformation in one chromosome or one pair of chromosomes.¹ I have pointed out elsewhere² that when the duplicate or triplicate condition occurs it might be reasonably supposed to have arisen through the same general change having taken place independently in two or three different chromosomes of the x series.

In an original mutation of this kind the new character of course forms a pair by contrast with the old unaltered character. If a single chromosome has undergone this change and the new condition is dominant, then a heterozygous mutant Aa will be produced having the new character but splitting in its offspring in a 3:1 ratio. This is the way in which *Oenothera rubricalyx* originated from *O. rubrinervis*, as I have shown elsewhere.¹

If now in the mutant race one or both members of a second pair, $a'a'$, of chromosomes undergoes a corresponding change, to $A'a'$, or $A'A'$ then we shall have duplicate factors AA' for the same character, and in the offspring of such individuals the new type and the original type would appear in the ratio of 15:1. A similar mutation in a third pair would give the triplicate condition with a ratio of 63:1.

It may be pointed out that this assumption of similar changes in different members of the x series of chromosomes is by no

¹ See Gates, R. Ruggles, 1915, "On the Origin and Behaviour of *Oenothera rubricalyx*," *Journ. of Genetics*, 4: 353-360.

² Gates, "The Mutation Factor in Evolution," p. 317, Macmillan, London, 1915.

means an improbable one. It does not assume that the chromosomes which underwent the change were alike, but merely that they were more nearly alike than the others of the series. That the chromosomes of the x series are actually differentiated there are many lines of evidence to show. One of the most recent is the fact, ascertained by Doncaster and Gray,¹ that in certain echinoderm crosses particular chromosomes swell up and form vesicles in the strange cytoplasm of the egg or of another species while other chromosomes exhibit no such effect. On the other hand, the materials of the chromosomes obviously possess many similarities which probably outweigh their chemical differences.

It may further be pointed out that if both members instead of one member of a pair of chromosomes underwent a change, say in a pollen mother cell, the only difference would be that four instead of two mutated germ cells would result, each of which might function in producing a mutant. It is almost impossible to determine whether one or both members of a pair of chromosomes underwent the change in any particular instance, but in either case the original mutant would be heterozygous, though continued inbreeding would produce ultimately a homozygous race, as in the case of *Æ. rubricalyx*. This is probably the history of Nilsson-Ehle's wheats which are duplicate or triplicate for the red color factor in their grains.

From the evidence obtained in F_2 and F_3 in crosses of various Swedish wheats having red kernels, with white-grained varieties, Nilsson-Ehle concludes² that while the varieties known as Sammet and Grenadier have 3 independent units for red, Extra-Squarehead has only one, since it gave (p. 67) only ratios approximating 3:1. In a later paper³ continuing this work the same author finds (p. 22) that Swedish Binkel wheat contains two factors for red. From one F_2 family he grew 94 F_3 families, with results which may be tabulated as follows:

¹ Doncaster, L., and Gray, J., 1913, "Cytological Observations on the Early Stages of Segmentation of *Echinus* Hybrids," *Quart. Journ. Micr. Sci.*, 58: 483-510, pls. 28-29.

² Nilsson-Ehle, H., 1909, Kreuzungsuntersuchungen an Hafer und Weizen. I. *Lunds Univ. Årsskrift.*, N.F., Afd. 2, Bd. 5, Nr. 2, pp. 122.

³ Nilsson-Ehle, H., 1911, "Kreuzungsuntersuchungen an Hafer und Weizen," II., *Lunds Univ. Årsskrift.*, N.F., Afd. 2, Bd. 7, Nr. 6, pp. 82.

TABLE I.

Expected Ratio of Families.		Totals.	Expectation.
7	40 families were constant red		
4	23 families split in the ratio 3 : 1	483 : 142	468.75 : 156.25
4	25 families split in the ratio 15 : 1	789 : 47	783.75 : 52.25
1	6 families were constant white		

It will be seen that the frequency of families is very close to expectation. The totals of the families containing a 15:1 ratio are also very close to expectation, but for the 3:1 families the agreement is not so good. The evidence seems sufficient, however, to justify the conclusion that two factors and two only are here concerned.

In the same way evidence is obtained (p. 25) to show that a certain pure line (0406) has in one case a single factor for red and in another case two factors. To use the terminology of Lang, the race is monomerous in one case and dimerous in another. In crosses between the 0406 race and 0234, which was also red,¹ ratios of 15:1 and 3:1 were obtained showing that two factors were present, one of which must have been derived from each parent. Hence the 0406 race must in this case have been monomerous. In crosses between 0406 and a white race, 15:1 ratios were again obtained, showing that the 0406 race is now dimerous. The genetic relationships of the strains used in these two crosses is not stated, but a simple explanation is that in the meantime the strain had undergone a second (invisible) mutation.

No explanation of the origin of this condition was offered. But there are at least two ways in which the dimerous condition may have been derived from the monomerous: (1) Through a mutation on the part of a second pair of chromosomes, (2) through a re-mating of the chromosome pairs. Later we shall compare the consequence of each of these methods of deriving the duplicate condition. In the first case the duplicate mutation is produced by a change very similar to that which produced the original mutant. In the second case the secondary change is a mechanical one, very different from the primary change which was probably chemical in nature.

¹ The results are given in *Ber. deut. bot. Gesells.*, 29: 65-69, 1911.

Another pure line of wheat (0290) was also found to be dimerous for the red factor in one case and monomeric in another. A race called 0501 was found to be probably trimerous like Swedish Sammet. Nilsson-Ehle considers it scarcely probable that in these two wheats the same three factors for red are present, and thinks that perhaps many more than three independent factors have to be reckoned with. There seems, however, no reason for such an assumption. It appears more probable that corresponding chromosomes undergo the same change in each case so that the factors are all homologous with each other, though of independent origin in the different races.

We may, therefore, account for the origin of the duplicate and triplicate "factors" for red in the Swedish wheats by assuming that successive mutations have occurred and that in each case the duplicate or triplicate condition has afterward become homozygous and stable through the repeated self-fertilization occurring in later generations.

Turning now to the history of *Oenothera rubricalyx*, it appears that the original monomeric condition has become dimerous in subsequent generations of culture. And it will be seen from the culture records that this has happened independently several times in different lines of descent.

It may be worth while first to recapitulate in briefest form the evidence for the original monomeric character of *O. rubricalyx*. The original mutant gave an F_1 offspring of 12 plants, 11 of which had red buds (R) and one green buds (r). Three of the former selfed produced F_2 families in which the ratios R:r were respectively 10:5, 14:6, and 33:11. The sum of these three families is 57:22 which is close to a 3:1 ratio and could not reasonably represent a 15:1 ratio, nor could either of the three ratios individually. Two plants descended from the F_2 family which yielded 33:11, a perfect 3:1 ratio were used to cross reciprocally with *O. grandiflora*, a green budded species of diverse habit. Since these plants were from a family which was obviously monomeric, the F_1 from the cross would either be all R (if the parent was homozygous) or R:r in equal numbers (if the parent was heterozygous). The numbers obtained were 30 R:28 r in one cross and 79 R:71 r in the other. Hence the family which gave

the ratio 33:11 was undoubtedly monomerous and up till that time a single mutation had taken place involving only one pair of chromosomes.

It was anticipated that the F_2 from *rubricalyx* \times *grandiflora* and its reciprocal would again yield 3:1 ratios but it was found that in fact there were other ratios as well, the chief of which now appear to be 2:1, 4:1, 5:1 and 15:1. In my discussion of these extensive results¹ I was at first inclined to attribute them to an effect of the *grandiflora* parent in modifying the frequency of inheritance of the R character, and to conclude that since the cross with *grandiflora* had obviously modified the red-bud character R by dilution in many cases, it must also have modified the frequency with which R would appear. I have since grown a large series of F_3 families, the results of which are published in detail elsewhere.² In the present communication a further analysis of these F_2 and F_3 ratios will be made, from which it appears that the unexpected ratios obtained in these generations are probably not an effect of the cross with *Æ. grandiflora*, but they result in part from the subsequent occurrence of duplicate mutations in *rubricalyx*. Other ratios, such as 5:1 cannot, however, be fully explained in this way.

In this connection it seems desirable to point out that in the inheritance of any character there are two features to be taken into consideration: (1) The nature of the character, and (2) the mechanism of its distribution in the germ cells. Mendelian writers frequently ignore the former, and biometrical writers vitiate their case when they take no account of the latter; but in a complete account of the inheritance of any character both must be considered. As a matter of fact, although crossing with *grandiflora* probably does not modify the mechanism of transmission of R, yet it does seriously and permanently modify the character itself in some cases, as I have shown in previous publications.

We may now consider the ratios R:r in the F_2 and F_3 of *Æ. rubricalyx* \times *grandiflora* and the reciprocal. A further study of

¹ Gates, R. R., 1914, "Breeding Experiments which Show that Hybridization and Mutation are Independent Phenomena," *Zeitschrift f. Abst. u. Vererb.*, 11: 209-279, Figs. 25.

² "The Mutation Factor in Evolution," pp. 254 ff.

these ratios makes it evident that they nearly all fall remarkably close to three or four ratios. So close is the fit that it seems probable that several ratios, such as 5:1, are significant as such, though at present no complete explanation of them can be offered. I was formerly inclined to regard some of these ratios as the expression of merely quantitative differences without

TABLE II.
 F_2 (*Oe. grandiflora* \times *rubricalyx*).

Ratios.	Expectation.	Agreement.	Conclusion.
68 : 16	$\begin{cases} 63.00 : 21 \\ 67.20 : 16.8 \end{cases}$	3 : 1 possible 4 : 1 very near	4 : 1
142 : 15	147.00 : 10	15 : 1	15 : 1
133 : 4	128.40 : 8.6	15 : 1	15 : 1
Total 275 : 19	275.60 : 18.4	15 : 1 perfect Hence 2 families 15 : 1 1 family 4 : 1	

F_2 (*Oe. rubricalyx* \times *grandiflora*).

(a) 66 : 13	$\begin{cases} 59.25 : 19.75 \\ 65.84 : 13.16 \end{cases}$	3 : 1	
(b) 45 : 14	44.25 : 14.75	5 : 1 perfect	5 : 1
(c) 47 : 3	Incomplete. In addition 9 dwarfs, 1 intermediate.	3 : 1 nearly perfect	3 : 1
(b) 134 : 44	133.50 : 44.5	3 : 1 perfect	3 : 1
(a) 67 : 13	$\begin{cases} 66.70 : 13.3 \\ 60.00 : 20 \end{cases}$	5 : 1 perfect	5 : 1
	$\begin{cases} 79.20 : 15.8 \\ 71.25 : 23.75 \end{cases}$	3 : 1 unlikely	
(a) 82 : 13	$\begin{cases} 89.00 : 6 \\ 76.70 : 15.3 \end{cases}$	5 : 1 very near	
	$\begin{cases} 69.00 : 23 \\ 86.25 : 5.75 \end{cases}$	3 : 1 very unlikely	5 : 1
(a) 77 : 15		15 : 1 " "	
$\begin{cases} 45 : 14 \\ 134 : 44 \end{cases}$		5 : 1 perfect	
(b) 179 : 58	177.75 : 59.25	3 : 1 unlikely	5 : 1
		15 : 1 very unlikely	
66 : 13			
67 : 13			
82 : 13			
77 : 15			
(a) 292 : 54	$\begin{cases} 288.30 : 57.7 \\ 259.50 : 86.5 \\ 324.40 : 21.6 \end{cases}$	5 : 1 very close	5 : 1
		3 : 1 unlikely	
		15 : 1 impossible ¹	
		Hence in F_2	
		2 families 3 : 1	
		4 " 5 : 1	
		0 " constant	

¹ By "impossible" is meant that the chances against this interpretation, taken in connection with the other results, are so great that for practical purposes it need not be considered.

F_3 (*Oe. grandiflora* \times *rubricalyx*).

Ratios.	Expectation.	Agreement.	Conclusion.
231 : 56	$\left\{ \begin{array}{l} 229.60 : 57.4 \\ 239.20 : 47.8 \\ 191.30 : 95.7 \\ 268.10 : 18.9 \end{array} \right.$	$\begin{array}{l} 4 : 1 \text{ very near} \\ 5 : 1 ? \\ 3 : 1 \text{ impossible} \\ 15 : 1 \text{ "} \end{array}$	4 : 1
237 : 56	$\left\{ \begin{array}{l} 234.40 : 58.6 \\ 244.20 : 48.8 \\ 219.75 : 73.25 \\ 274.70 : 18.3 \end{array} \right.$	$\begin{array}{l} 4 : 1 \text{ very near} \\ 5 : 1 ? \\ 3 : 1 \text{ improbable} \\ 15 : 1 \text{ impossible} \end{array}$	4 : 1
Total 468 : 112	$\left\{ \begin{array}{l} 464.00 : 116 \\ 483.30 : 96.7 \\ 435.00 : 145 \\ 543.75 : 36.25 \end{array} \right.$	$\begin{array}{l} 4 : 1 \text{ very near} \\ 5 : 1 \text{ improbable} \\ 3 : 1 \text{ improbable} \\ 15 : 1 \text{ impossible} \end{array}$	4 : 1
Hence F_3 (<i>grandiflora</i> \times <i>rubricalyx</i>)			
2 families 4 : 1			
Also			
4 families constant R			
3 " constant r			
2 " intermediate			
in pigmentation of buds.			

 F_3 (*Oe. rubricalyx* \times *grandiflora*).

57 : 31	$\left\{ \begin{array}{l} 58.70 : 29.3 \\ 66.00 : 22 \\ 82.50 : 5.5 \end{array} \right.$	$\begin{array}{l} 2 : 1 \text{ very near} \\ 3 : 1 ? \\ 15 : 1 \text{ impossible} \end{array}$	2 : 1
2 : 3		?	
22 : 6	21.00 : 7	3 : 1 very near	3 : 1
112 : 69	$\left\{ \begin{array}{l} 120.70 : 60.3 \\ 135.75 : 45.25 \end{array} \right.$	$\begin{array}{l} 3 : 1 \text{ impossible} \\ 2 : 1 \text{ near?} \end{array}$	2 : 1 ?
12 : 2		?	
182 : 29	$\left\{ \begin{array}{l} 176.00 : 35 \\ 168.80 : 42.2 \\ 197.80 : 13.2 \end{array} \right.$	$\begin{array}{l} 5 : 1 \text{ near} \\ 4 : 1 \text{ impossible} \\ 15 : 1 \text{ impossible} \end{array}$	5 : 1
4 : 2		?	
55 : 2	53.40 : 3.6	15 : 1 close	15 : 1
37 : 2	36.60 : 2.4	15 : 1 perfect	15 : 1
72 : 6	73.10 : 4.9	15 : 1 very near	15 : 1
59 : 1	56.25 : 3.75	15 : 1 not very close	15 : 1
Total 223 : 11	219.40 : 14.6	15 : 1 very close	15 : 1
79 : 37	$\left\{ \begin{array}{l} 77.30 : 38.7 \\ 87.00 : 29 \\ 108.75 : 7.25 \end{array} \right.$	$\begin{array}{l} 2 : 1 \text{ very near} \\ 3 : 1 ? \\ 15 : 1 \text{ impossible} \end{array}$	2 : 1 ?
5 : 3		?	
80 : 34	$\left\{ \begin{array}{l} 85.50 : 28.5 \\ 76.00 : 38 \end{array} \right.$	$\begin{array}{l} 3 : 1 \text{ near} \\ 2 : 1 \text{ nearer} \end{array}$	2 : 1 ?
Hence in F_3 <i>rubricalyx</i> \times <i>grandiflora</i>			
Probably			
4 families 2 : 1 ratio			
1 family 3 : 1 "			
1 " 5 : 1 "			
4 families 15 : 1 "			
Also			
2 families constant for R			
8 " constant for r			
1 family intermediate			
186 plants.			

more precise significance, but particularly the ratios 5:1 and 15:1 in addition to 3:1 fall so closely into definite categories that the probability of there all being significant is great. The foregoing table contains the F_2 and F_3 ratios for the various families.

Considering these data as a whole, the ratios nearly all fall remarkably close to whole numbers. In the first column of Table II. are given the actual ratios obtained, in the second column the expectation for different ratios, and in the third column the conclusion as to the ratios probably represented in each case. Many of the families are larger than these of Nilsson-Ehle, and in general they appear to fit the various ratios more closely.

Considering first the F_2 families, it will be seen that those whose ratios are 3:1 or 15:1 are in perfect or almost perfect accord with expectation. This being the case, it seems probable that the 5:1 and 4:1 ratios obtained are significant as such, and in any case they cannot be considered merely wide departures from 3:1. Of the 5:1 ratios the first, second, and fourth are in perfect agreement with 5:1 while the other one is very close, as is also the sum of these four families (292:54). The significance of these facts is further heightened by the fact that three of these four families (the first, third and fourth) are derived from selfing different flowers of the same F_1 plant. This is shown in my original record of these experiments,¹ and it almost forces the conclusion that in this particular plant as well as others giving similar ratios, R gametes were being produced with greater frequency than r gametes in the ratio 5:3. There is, however, another explanation which will be considered later.

In the results of Nilsson-Ehle, on the other hand, the ratios do not fall clearly into such intermediate categories but tend to form a continuous series of ratios as Kajanus pointed out. Thus in one series of crosses² between black and white glumes involving only monohybrid ratios, the F_2 ratios in the 13 families actually range from 2.2:1 (323:144) to 4.1:1 (230:56), yet the total (2468:795) is fairly close to 3:1. One of these families contained 86

¹ *Zeitschr. f. Abst. u. Vererb.*, 11, p. 236.

² "Kreuzungsuntersuchungen," I, p. 18.

black (B): 22 white (B). F_3 offspring were grown from each of these 108 plants and the results showed their composition to have been as follows: 36 BB:50Bb:22 bb. From this result Nilsson-Ehle concludes that there was a preponderance of "black" gametes over "white" ones. But a series of F_3 families in another cross gave the reverse condition, 26 BB:60Bb: 33 bb, from which the conclusion is drawn that white gametes were here more numerous than black ones. Even though these results offset each other yet they cannot be referred merely to chance fluctuations in ratios. But no further explanation of them was offered. It will be shown later that in my crosses of *Æ. rubricalyx* and *Æ. grandiflora* these deviating ratios do not offset each other but are all consistent with the hypothesis that R gametes are being produced with greater frequency than r gametes.

Returning now to Table II. the first ratio (68:16) is not a very bad fit for 3:1, although exceedingly close to 4:1. It might easily pass for 3:1 without further comment were it not for the fact that two other ratios in this table are in very close agreement with 4:1 while they depart very widely from 3:1. The sum of these two ratios (468:112) is very close to 4:1 while it is highly improbable as a 3:1 ratio, the more so since the actual 3:1 ratios are almost in precise agreement with expectation.

Among the three F_2 families from *Æ. grandiflora* \times *rubricalyx*, two show a 15:1 ratio and one a 4:1 or perhaps a 3:1 ratio. Hence it might be supposed that the *rubricalyx* plant which was used as pollen parent, already possessed duplicate factors for red. But this was not the case. That plant was in fact heterozygous for a single factor, since when crossed with *grandiflora* it gave an F_1 of 79R : 71r, which is as near to equality as could be expected. The full history of the *rubricalyx* individuals used for this and the reciprocal cross has been given in pedigree form in another paper,¹ to which reference should be made. It may be said that in both cases they are descended from the family which contained 33R : 11r. One member of this family was pollinated by *nanella* and produced a family of 42 plants. One of the latter (No. IV., 2), which was a perfect *rubricalyx* in appearance but

¹ Gates, *Zeitschr. f. Abst. u. Vererb.*, 11: opp. p. 216 and on p. 217.

carried dwarfing latent, was used to pollinate *Æ. grandiflora*. Since the F_2 offspring of this cross gave 15:1 ratios in two families, while the F_1 was a 1:1 ratio, duplicate mutations must have intervened between these two generations. The two plants which were the parents of the families containing 142 R: 15 r and 113 R: 4 r respectively must have possessed the duplicate factor in all their germ cells, so that they were heterozygous for R and R'. Their composition might then be written RrR'r'.

As pointed out earlier in this paper, such a condition might have arisen (a) through the transformation of a chromosome belonging to a second pair, (b) through an exchange of mates on the part of two pairs of chromosomes. We may now examine the comparative credibility of these two alternatives. There are certain difficulties with either hypothesis, one of which is that the transformation from the monomeric to the dimerous condition, whether effected by chemical or mechanical means, must apparently have taken place early in the ontogeny, before definitive germ cells are formed. The alternative hypothesis would be that all the germ cells had undergone the transformation simultaneously and independently, which one cannot believe possible.

There is, however, one consideration which makes it appear probable that the duplicate condition for R is not usually arrived at through a transformation of a new chromosome, but rather through a redistribution of the chromosomes. The 15:1 ratio can only be obtained from an RrR'r' parent, in which both duplicate factors are heterozygous. It would therefore be necessary to assume when a 15:1 family is derived from a 3:1 family, that a chromosome belonging to a new pair had undergone a chemical transformation while its mate and the mate of the original modified chromosome were unaffected, *i. e.*, that the condition Rrr'r' became altered directly to RrR'r'. This is very unlikely. On the other hand, as I showed long ago,¹ the chromosomes in *Ænothera* are very loosely paired during the reduction division, and moreover irregular chromosome distributions have been shown to occur at this time (as in the production of *Æ. mut. lata*). I also (*l. c.*) pointed out the probability

¹ "A Study of Reduction in *Ænothera rubrinervis*," *Bol. Gazette*, 46: 1-34, pls. 3, 1908.

that exchanges of chromosomes of different pairs but without change in number would take place under these conditions. By such a mismating or exchange of mates on the part of two chromosome pairs, a plant which was homozygous (RR) for one factor would give rise to plants which were heterozygous for duplicate factors ($RrR'r'$). This is then what has probably occurred in the cases where plants from a 3:1 family have given rise to 15:1 ratios. The frequency with which such mismating occurs in *Ænothera* may thus be estimated.

It is known that the chromosomes of *Ænothera* are in pairs (doubtless of paternal and maternal origin) throughout the somatic divisions, and the paired arrangement is probably a feature of the first mitosis after fertilization. If, then, a plant which would have been homozygous for a single factor (RR) becomes transformed into one which is heterozygous for duplicate factors ($RrR'r'$) and so gives a ratio 15:1 in its offspring, the most likely assumption is that *at the time of fertilization* the two R chromosomes, instead of becoming paired with each other, each paired with another (r) chromosome. Hence in this case the regrouping of chromosomes probably occurred not during meiosis where it would have to occur simultaneously in all the germ cells, but as a feature of fertilization or the first mitosis of the embryo. It will be shown later, however, that mismatings of the chromosome pairs probably also occur during meiosis and so modify the 3:1 ratio. To sum up, it appears that when a 15:1 family is derived directly from a plant in a 3:1 family, the remating of the chromosomes must have occurred at fertilization or soon afterwards; but when, for example, a 4:1 or a 5:1 family is derived from a 3:1 family, this may be accounted for by a certain amount of remating of chromosomes during meiosis.

The method above described will also apply to the origin of duplicate and triplicate factors in wheat and is perhaps more probable than the successive chemical transformation of different chromosomes. There is, however, a method of testing between these two possibilities. If the duplicate condition arises through a regrouping of the chromosome pairs, then, as has been mentioned, a race or a plant homozygous (RR) for one factor will give rise to a plant heterozygous for two factors

($RrR'r'$). On the other hand, if the chemical transformation of a fresh chromosome takes place in a homozygous monomeric plant (RR), then the dimerous individual derived from such a monomeric plant should have the constitution $RRR'r'$.¹ It would be possible to determine between these two alternatives by breeding tests. If the constitution of the plant is $RrR'r'$ its offspring should give a 15:1 ratio. If it is $RRR'r'$ they would all be red in F_1 and F_2 . But plants having the former formula could also be produced by the mismating of chromosome-pairs during meiosis in RR plants.

If we now return to the table (p. 210) and examine the F_2 from the reciprocal cross (*rubricalyx* \times *grandiflora*) we find a total absence of 15:1 ratios, showing that not only was the *rubricalyx* parent of this cross monomeric but its offspring remained so. The parent of this cross was a member (No. IV., 8) of the monomeric family 33:11. As will be seen from the table, two of the F_2 families from *rubricalyx* \times *grandiflora* gave perfect or almost perfect 3:1 ratios. Four others gave 5:1 ratios, three of which were perfect and the other very close to expectation as already pointed out. I have at present no further explanation of these 5:1 ratios to offer, but it seems probable that their significance will later become apparent.

Ratios more or less in excess of 3:1 could be obtained from plants homozygous for one factor, if there was a tendency for mismating of the chromosomes in meiosis. But this will not account for the definiteness of the 5:1 ratios obtained.

Turning to the F_3 of *grandiflora* \times *rubricalyx* the full data are given in my book (p. 255). Four families were constant for R , 3 constant for r , 2 families numbering respectively 283 and 20 plants bred true to an intermediate condition, and 2 families split in the ratio 4:1, as shown in the table (p. 211). The excess of R 's in the last two families is a significant excess over 3:1, however it is brought about.

In the F_3 of *rubricalyx* \times *grandiflora*, four families give ratios nearest 2:1, one family near 3:1, one near 5:1 and four very close to 15:1. Whatever the significance of the 2:1 and 5:1 ratios in

¹ We have already found it highly improbable that a plant Rr could be directly transformed chemically into $RrR'r'$, since we should anticipate that the chromosome r would undergo a mutation before the chromosome r' .

these families, the appearance of 15:1 ratios in the F_3 of this cross is of much interest, since the F_2 contained no families which could reasonably be construed as containing duplicate factors, except the one having the incomplete ratio 47:3. Reference to the pedigree numbers¹ shows that the first two are derived from the F_2 family No. 60 in which the ratio is doubtful, the third is derived from selfing a plant in the F_2 family No. 62, and the fourth from selfing one in family No. 63. In these two families the ratios were respectively 67:13 and 82:13, both of which are shown (p. 210) to be very near 5:1 ratios. The appearance of these 15:1 ratios in F_2 from 5:1 families can be explained if we assume that independent duplicate mutations have occurred in the F_2 families 60, 62, and 63. This must happen as previously outlined, through a plant which is homozygous for one factor giving rise to a plant which is heterozygous for two; or in other words, through the rearrangement of a pair of homologous chromosomes so that they belong to different pairs.

Another point which will be explained by the present hypothesis is the difference in the depth of color in homozygous red-budded races. Thus in the F_3 families 93 and 95,² containing respectively 280 and 312 plants, the latter were constantly darker red than the former. The latter family was doubtless homozygous for duplicate factors ($RRR'R'$), or at least $RRR'r'$, since the family from which it was derived yielded 15:1 ratios. The former family was on the other hand probably homozygous for a single factor (RR) and hence not so densely red-pigmented.

It will thus be seen that in several instances 15:1 families have been obtained from the offspring of 3:1 or 5:1 families. All such cases can be explained by assuming that a duplicate mutation has intervened. The original mutation by which deep red buds in *Oenothera* first appeared is an extremely rare occurrence, having occurred but once in all cultures of *Oenothera*. When, however, a chromosome has once undergone this change it is reasonable to suppose that other chromosomes in the same nucleus could without difficulty take on an analogous transformation. The whole mechanism is, however, at hand in the

¹ See "The Mutation Factor in Evolution," p. 256.

² *L. c.*, p. 255.

meiotic divisions, for transforming the original 3:1 ratio into a 15:1 by merely redistributing the chromosome pairs.

In concluding this paper it is desirable to compare the related but different results recently obtained by Honing,¹ with two varieties of *Canna indica* which are naturalized in Sumatra. One variety has green leaves while in the other the leaves have a broad red margin.

From the offspring of plants of the latter variety Honing obtained ratios red: green of 3:1, 9:7 and 27:37. The same ratios were obtained in crossing the two varieties. These ratios are accounted for by the hypothesis that the coöperation of three "factors" is necessary to produce the red margin. If these are located in chromosomes belonging to three different pairs, then the resulting ratio should be 27 red: 37 green, since the character can only appear in the presence of all three factors A, B, C. On the other hand, if all three factors are located in the same chromosome a 3:1 ratio would be obtained, while if two of them were in one chromosome and the third in a chromosome of a second pair, the ratio would be 9:7.

It was found that in certain cases plants in families having a 3:1 ratio gave rise in the next generation to a 9:7 or 27:37 family. In such cases one may assume that a mutation has taken place resulting in a redistribution of the determiners, the three which were present in one chromosome being rearranged so that they are in chromosomes belonging to two or three different pairs. So far as I am aware, this is the first experimental evidence that an actual rearrangement of the chromomeres in the chromosomes is one of the kinds of change which the nucleus may undergo, the case being somewhat different from Morgan's well-known phenomena of "crossing over" in *Drosophila*. Further experiments are necessary to test the nature of this evidence for the occurrence of mutations in which such a rearrangement of the nuclear material can take place.

SUMMARY.

Nilsson-Ehle was the first to show that duplicate and triplicate factors for red are present in certain strains of wheat. He

¹ Honing, J. A., 1915, "Kreuzungsversuche mit *Canna*-Varietäten," *Rec. Trav. bot. Néerlandais*, Vol. 12: Livr. 1, pp. 26.

found, moreover, that the same strain may be in one case monomeric and in another case dimerous for this character; and that while, for example, Grenadier wheat possessed three independent units for red, Extra-Squarehead possessed only one. The origin of the original "factor" for red may be accounted for in the wheats as in *Ænothera rubricalyx*, through the chemical transformation of one chromosome or a pair of homologous chromosomes. The duplicate condition for the character R may have arisen (1) through a chemical mutation in a second pair of chromosomes, (2) through a re-mating of the chromosomes (RR) forming a homozygous pair. The latter method is for various reasons the more probable.

Although the original *Ænothera rubricalyx* was a monohybrid and continued so for at least two generations, yet in subsequent generations involved in crosses with *Æ. grandiflora*, 15:1 or di-hybrid ratios were derived from the offspring of members of 3:1 families. This can best be accounted for by supposing that in a plant (RR) homozygous for one factor, a re-grouping of the chromosome pairs occurred. This re-grouping involves merely an exchange of mates on the part of the chromosomes RR so that they now belong to different pairs. The formula for the plant may now be written $RrR'r'$, *i. e.*, the plant is heterozygous for two independent units for red and its offspring will give a 15:1 ratio.

The second mutation, producing the duplicate condition for R, is thus probably a purely mechanical process, while the original mutation which produced the "factor" R is a chemical change of wholly different nature. It is possible that in some cases the duplicate and triplicate conditions also arise through the chemical transformation of additional chromosomes.

When a 15:1 family arises from a 3:1 or 5:1 family, as has happened several times in *Æ. rubricalyx* hybrids, it is necessary to assume that the regrouping or remating of chromosome pairs which led from the monohybrid to the dihybrid condition, took place at fertilization, or at any rate early in the ontogeny, and is then handed down to the germ cells by mitosis. The chromosomes are known to be paired in the somatic divisions, and it seems probable that the manner of pairing set up in fer-

tilization continues in this case throughout the ontogeny, though this is not true for all organisms. Otherwise it would be necessary to assume that when a plant in a 3:1 family gives rise to a 15:1 family all its germ cells have simultaneously undergone a mis-mating of the chromosome pairs during meiosis, a highly improbable event.

In the F_2 and F_3 hybrids of *Æ. rubricalyx* and *Æ. grandiflora*, in addition to 3:1 and 15:1 ratios, 2:1, 4:1 and 5:1 ratios occur. The 5:1 ratios at least seem to be significant, indicating that R and r gametes are regularly being produced in the ratio 5:3, or that a certain amount of re-grouping of the R chromosomes is regularly occurring during meiosis.